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A Murine Model for the Study of *Chlamydia trachomatis* Genital Infections during Pregnancy

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Pregnant BALB/c mice were inoculated intravaginally on day 5 of gestation with the *Chlamydia trachomatis* mouse pneumonitis biovar. Animals that received 10^5, 10^6, or 10^7 inclusion-forming units (IFU) of *C. trachomatis* delivered prematurely on days 15 to 16 of gestation. A focal inflammatory infiltrate was observed in the wall of the uterus on the day 14 of gestation in animals inoculated with 10^5 IFU. In this group of mice, immunohistochemical analysis showed chlamydial inclusions in the endometrium and fetal membranes.

Infant mortality rates in the United States continue to be higher than those of most industrialized countries and have recently increased (9, 13, 18). These high infant mortality rates are mainly due to high rates of premature birth and associated low birth weight. The magnitude of this problem is such that recently, Hillier et al. (13) concluded that preterm delivery, low birth weight, and neonatal mortality are the most important problems in obstetrics. Determinants that affect low birth weight include genetic, social, environmental, and behavioral factors. Among these, infections of the genital tract are considered to account for up to 40% of preterm births and thus are probably the most significant contributors to high infant mortality rates (20). Organisms that have been associated with this problem include, among others, *Chlamydia trachomatis*, *Gardnerella vaginalis*, *Mycoplasma hominis*, *Neisseria gonorrhoeae*, *Streptococcus agalactiae*, *Trichomonas vaginalis*, *Ureaplasma urealyticum*, and other pathogens involved in bacterial vaginosis (9, 13).

C. trachomatis is one of the most common sexually transmitted pathogens in the Western world (4, 10, 24). Several studies over the last two decades have attempted to determine the impact that a *C. trachomatis* genital infection has on pregnancy outcome. Some of these studies found maternal and fetal morbidity and mortality associated with both acute and past chlamydial infections, while others did not confirm these data (2, 5, 6, 8, 12, 14, 16, 19). These contradictory results are not surprising considering the problems encountered in conducting these types of studies in humans, where assessment of a chlamydial infection is very difficult. Thus, only in an animal model can we start to characterize the role that a *C. trachomatis* infection may play in the outcome of pregnancy and on the mechanisms that may be involved in the pathogenesis of the disease. In this study, we describe a new murine model in which we determined the effect that an acute chlamydial genital infection during gestation has on pregnancy outcome.

C. trachomatis mouse pneumonitis (MoPn) biovar (strain Nigg II; American Type Culture Collection, Rockville, Md.) was grown in HeLa 229 cells (American Type Culture Collection), and elementary bodies (EB) were purified and stored in 0.2 M sucrose–20 mM sodium phosphate (pH 7.2)–5 mM glutamic acid (SPG) as previously described (3, 17). Eight- to 9-week-old female and proven breeder male BALB/c ($H\text{-}2^d$) mice were purchased from Charles River (Wilmington, Mass.). Mice received normal diet and water ad libitum and were kept in isolation cubicles at a constant temperature of 24°C, with a cycle of 12 h of fluorescence light and 12 h of darkness. Groups of four female mice were housed with one male mouse and examined every morning for the presence of a vaginal plug as an indication of successful mating. When a vaginal plug was seen, the mouse was marked, weighed, and placed in a separate cage. The day the vaginal plug was observed was considered day 0 of gestation. Mice were inoculated intravaginally with *C. trachomatis* MoPn in 20 μl of SPG on day 5 of gestation with doses ranging from 10^1 to 10^7 inclusion-forming units (IFU) (7, 17). Three control groups were included in this study. The first control group received mock-infected HeLa 229 cell extracts in 20 μl of SPG processed in the same way as purified EB. The second group was inoculated with 10^5 *C. trachomatis* IFU that had been heat killed (HK) in 20 μl of SPG. A third control group was inoculated with 20 μl of SPG. Mice were examined and weighed daily to ascertain the progress of the pregnancy starting on day 10 of gestation. Within 24 h after birth, pups were weighed and body lengths were recorded. For histopathological studies, 15 fetuses from animals inoculated with 10^5 IFU of *C. trachomatis* MoPn and 12 controls from mice injected with HeLa 229 cell extracts were examined on day 14 of gestation. The uterine horns were fixed with the fetuses in situ, and tissue sections stained with hematoxylin and eosin (H&E). For immunohistological (IHC) analysis, staining with a rabbit anti-*C. trachomatis* MoPn serum followed by a biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, Calif.) was used to detect chlamydial inclusions, and the sections were counterstained with hematoxylin (17). To confirm that the staining was specific for *C. trachomatis* MoPn, normal rabbit serum was used as a control. For statistical analyses, differences between the control and infected animals in the occurrence of prematurity and birth rates were determined by Fisher's exact test. Differences between groups in body weight and body length were compared by unpaired Student's *t* test. The protocol was approved by the University of California, Irvine, Institutional Animal Care and Use Committee.

Mice infected with 10^5, 10^6, and 10^7 IFU of *C. trachomatis* showed signs of lethargy, hunched posture, and ruffled hair starting day 14 of pregnancy. Animals inoculated with 10^1, 10^3, or 10^4 IFU and the controls inoculated with HK *C. trachomatis*, HeLa cell extracts, and SPG showed no clinical abnormalities. All mice inoculated with 10^6 or 10^7 IFU of *C. trachomatis* delivered prematurely (Table 1). The mean gestation times at which delivery occurred for these groups were days 16.3 and

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TABLE 1. Effects of different *C. trachomatis* MoPn inocula on pregnancy outcome

Inoculum dose/mouse	No. of pregnant mice that delivered		Mean no. of babies born/ pregnant mouse ± 1 SD	Mean no. of gestation days at time of delivery		Characteristic of conceptus at birth[a]	
	Prematurely/total (%)	At term/total (%)		Premature	Normal	Mean wt (g) ± 1 SD	Mean length (cm) ± 1 SD
C. trachomatis MoPn							
10^7 IFU	7/7 (100)[b]	0/7 (0)[b]	NA[c]	16.4	NA	NAM[d]	NAM
10^6 IFU	4/4 (100)[b]	0/4 (0)[b]	NA	16.3	NA	NAM	NAM
10^5 IFU	12/13 (92.3)[b]	1/13 (7.7)[b]	5[f]	15.8	19.0	1.43 ± 0.08	2.70 ± 0.07
10^4 IFU	0/7 (0)	6/7 (85.7)[e]	4.1 ± 2.4	NA	19.6	1.48 ± 0.19	2.82 ± 0.12
10^3 IFU	0/4 (0)	4/4 (100)	6.3 ± 1.7	NA	19.5	1.44 ± 0.17	2.75 ± 0.11
10^1 IFU	0/3 (0)	3/3 (100)	5.3 ± 1.2	NA	19.6	1.62 ± 0.16	2.87 ± 0.15
10^5 HK *C. trachomatis* MoPn	0/6 (0)	6/6 (100)	6.3 ± 1.9	NA	19.3	1.33 ± 0.23	2.65 ± 0.16
HeLa cell extract	0/22 (0)	22/22 (100)	5.3 ± 1.3	NA	19.3	1.55 ± 0.21	2.80 ± 0.25
SPG	0/9 (0)	9/9 (100)	5.4 ± 1.5	NA	19.3	1.39 ± 0.19	2.76 ± 0.18

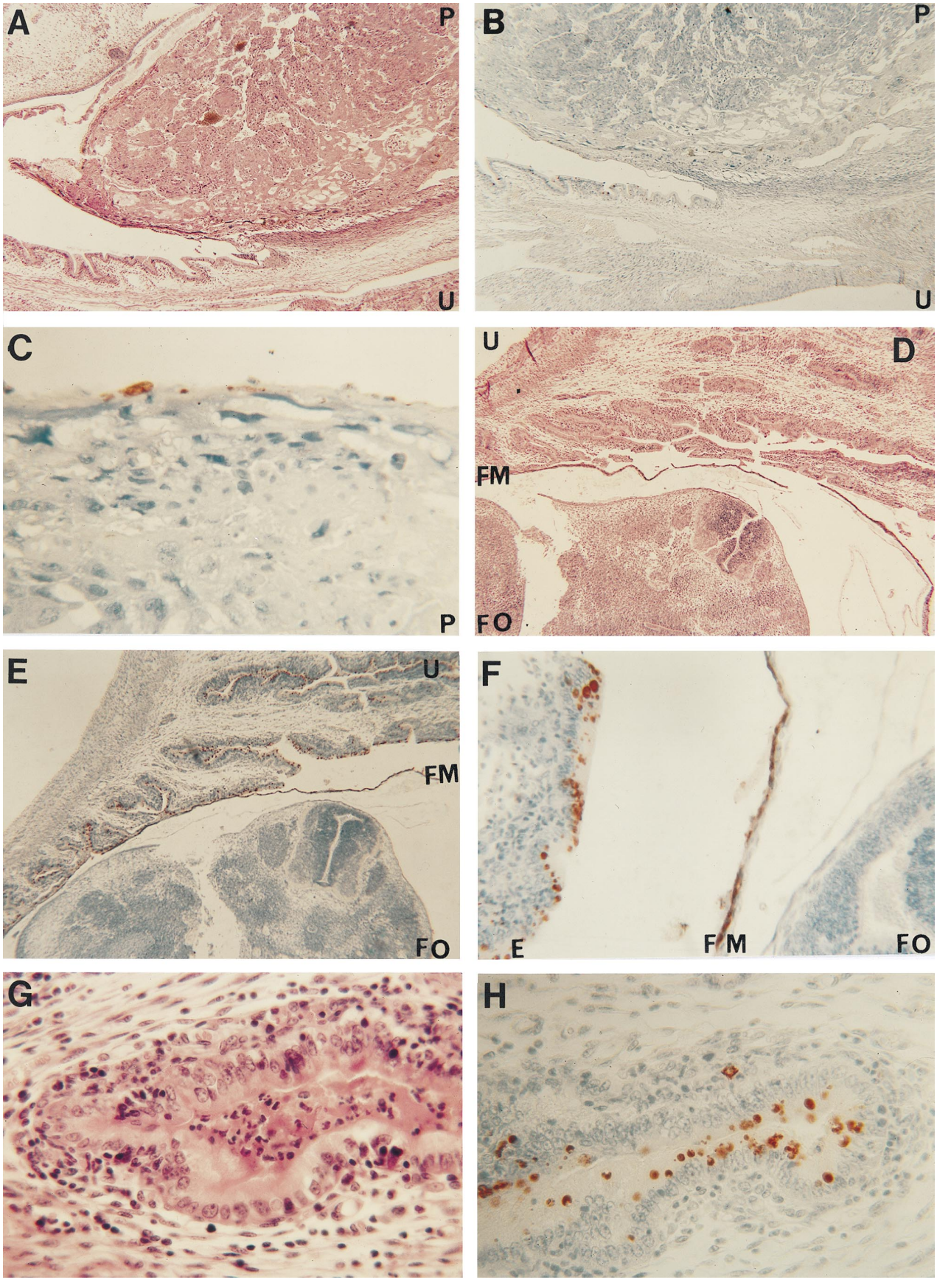
[a] Body length and weight were measured within 24 h after birth.
[b] $P < 0.05$ by Fisher's exact test compared with the HeLa cells extract or HK *C. trachomatis* MoPn-inoculated group.
[c] NA, not applicable.
[d] NAM, not available for measurement.
[e] One mouse died before delivery.
[f] Babies delivered from one mother.

16.4, respectively. Of the 13 mice inoculated with 10^5 IFU, 12 (92.3%) delivered prematurely and only 1 delivered on day 19 of pregnancy. The mean gestation time for delivery to occur in this group was day 15.8. In contrast, the mean delivery time for the mice receiving 10^4, 10^3 and 10^1 IFU was day 19.6 or 19.5. All control mice inoculated with HK *C. trachomatis* MoPn, HeLa 229 cell extracts, or SPG delivered normal pups. The mean delivery date for the three control groups was day 19.3. Animals born prematurely were cannibalized by their mothers and so were not available for measurement. The mean numbers of babies born per pregnant mouse were similar for all groups. No significant differences were observed in the mean body weight or length between the control animals and those inoculated with *C. trachomatis* MoPn that were born on day 19 or 20 of gestation.

In a majority (12 of 15) of the fetuses processed for histopathology from the pregnant mice inoculated with *C. trachomatis* MoPn, a mild focal acute inflammatory reaction consisting mainly of polymorphonuclear leukocytes with scattered mononuclear inflammatory cells, including some plasma cells, was observed in the maternal uterine wall (Fig. 1). In 3 of the 15 specimens studied, the inflammatory response was more severe. No inflammatory reaction was detected in the fetal tissues. IHC staining with a specific chlamydial rabbit polyclonal antibody revealed *C. trachomatis* inclusions in the maternal endometrium, in the splanchnopleure of the yolk sac, and in the periplacental bilaminar omphalopleure (15). Chlamydial inclusions were more numerous in the tissues in which there was a severe inflammatory reaction. No chlamydial inclusions were found in the zone of giant cells, trophospongium, the labyrinth, or the chorionic plate of the chorioallantoic placenta. Similarly, no chlamydial inclusions were detected in the amnion or in the fetal organs. Fetal and maternal tissues from 12 control fetuses had no inflammatory response, and no chlamydial inclusion were detected.

The effects of a *C. trachomatis* infection on pregnancy remain controversial (2, 5, 6, 8, 12, 14, 16, 19). Most likely, depending on the infecting inoculum, time of gestation, and susceptibility of the host, a wide variety of clinical manifestations ranging from asymptomatic infection to termination of pregnancy may occur. In a preliminary report, Spiliopoulou et al. (21) indicated that intravenous inoculation of Swiss mice on day 11 of gestation with doses ranging from 10^5 to 10^7 IFU of the *C. trachomatis* serovars E and L1 resulted in a reduced number of infant mice. A strong colonization of the placenta was observed, whereas colonization of the fetus was less extensive. Tuffrey et al. (23) inoculated intraperitoneally, or intravenously and intravaginally, TO mice with *C. trachomatis* serovar E either 14 days before detection of a vaginal plug or from 1 to 9 days thereafter. *C. trachomatis* was isolated from the placental disk in approximately 25% of the mice but not from fetal tissue or from maternal spleens. However, litter size and percentage of fetuses dying were not significantly different between the test and control animals. Thus, the conclusion from these experiments was that *C. trachomatis* did not affect pregnancy outcome and did not cross the placenta. As indicated by Tuffrey et al. (23), the main weakness of the model is that there is no evidence that intravaginal inoculation with the human *C. trachomatis* serovars in mice nonpretreated with progesterone results in infection of the upper genital tract. In fact, even in mice pretreated with progesterone, the ability of the human serovars of *C. trachomatis* to cause significant upper genital infection has been questioned (22). With the *C. trachomatis* MoPn biovar, on the other hand, we have shown that intravaginal inoculation, without pretreatment with progesterone, can result in salpingitis and infertility (7). Thus, the mouse

FIG. 1. (A to C) Histological section at the placental site of insertion stained with H&E (A [magnification, ×30]) and an IHC stain for *C. trachomatis* (B [×30] and C [×250]). The overall architecture of the placenta and the uterine wall is well preserved (A). Chlamydial inclusions can be detected in the endometrium (B) and in the periplacental bilaminar omphalopleure (B and C). (D to F) Section of the fetus and uterine wall stained with H&E (D [×30]) and an IHC stain for *C. trachomatis* (E [×30] and F [×160]). Fetal tissues appear normal and at a developmental stage corresponding to 14 to 15 days of gestation (D). Chlamydial inclusions can be observed in the endometrium and in splanchnopleure of the yolk sac (E and F). (G and H) Uterus stained with H&E (G [×250]) and the IHC stain for *C. trachomatis* (H [×250]). A moderate acute and chronic inflammatory reaction (G) and multiple chlamydial inclusions (H) can be observed in the endometrium. Abbreviations: E, endometrium; FM, fetal membranes; FO, fetal organs; P, placenta; U, uterus.



serovar in this respect more closely parallels a human genital infection, and as a result, we should be able to better assess the effects of a *C. trachomatis* infection on pregnancy outcome. Here, using this model, we have shown that *C. trachomatis* MoPn inoculated intravaginally on day 5 of gestation infects the endometrium and the membranes of the yolk sac, resulting in early termination of pregnancy. This is not surprising since chlamydial endometritis commonly occurs during a genital infection and the ability of *C. trachomatis* to infect amniotic cells has been demonstrated in vitro (11). Most likely, the fetal membranes were affected following infection of the endometrium. It is possible that the direct damage to the fetal membranes resulting from the infection, in combination with the endotoxin activity of the chlamydial lipopolysaccharide, is a significant factor in the premature termination of pregnancy.

In conclusion, we have shown that a genital infection early in gestation with a high chlamydial inoculum can result in premature termination of pregnancy, while a low inoculum does not appear to affect the course of gestation. We realize that due to the anatomical and physiological differences between a human and a murine pregnancy, there are limitations in this model. However, mice have been successfully used to characterize some of the effects of bacteria on pregnancy outcome (1). Thus, we think that this model could be very helpful for gaining an understanding of the possible effects and pathogenesis of chlamydial infections during gestation and for assessing the possibilities for developing preventive measures.

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